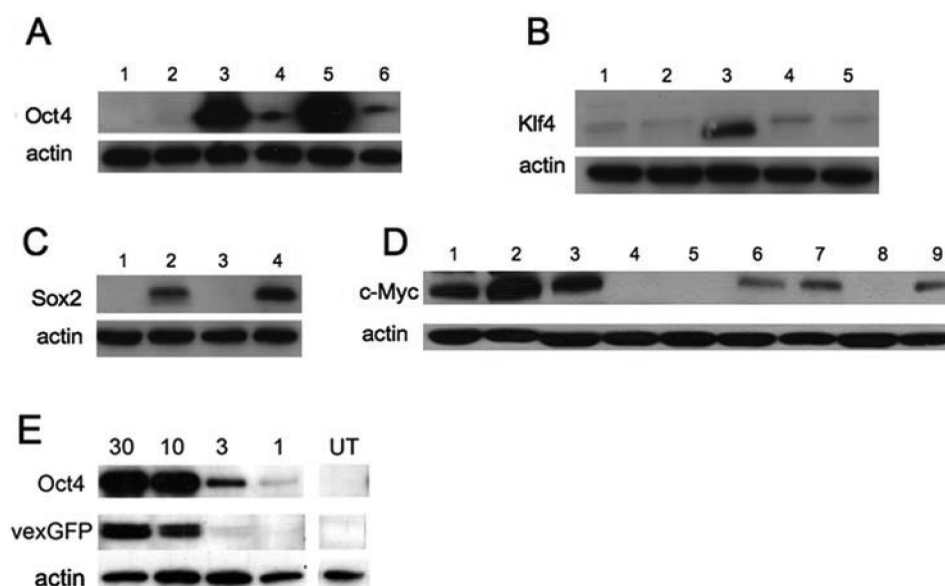


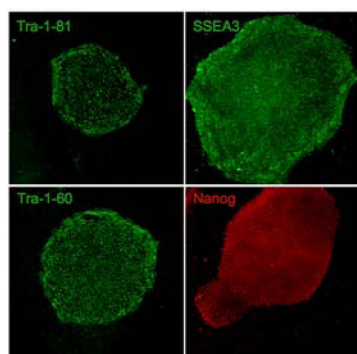
# Supporting Information

Papapetrou et al. 10.1073/pnas.0904825106

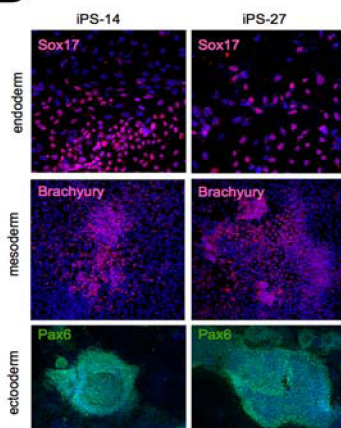


**Fig. S1.** Immunoblots of HeLa and MRC-5 cells transduced with the vectors shown in Fig. 1A. (A) 1, mock-transduced HeLa; 2, HeLa transduced with vector encoding vexGFP alone; 3, HeLa transduced with vexGFP-Oct4-encoding vector at MOI 5; 4, HeLa transduced with all 4 reprogramming vectors shown in Fig. 1A at MOI 1; 5, NT2D1 human embryonic carcinoma (hEC) cell line; 6, H9 hESC. (B) 1, mock-transduced HeLa; 2, HeLa transduced with vector encoding mCherry alone; 3, HeLa transduced with mCherry-Klf4-encoding vector at MOI 5; 4, NT2D1 hEC; 5, H9 hESC. (C) 1, mock-transduced HeLa; 2, HeLa transduced with mCitricine-Sox2-encoding vector at MOI 5; 3, HeLa transduced with vector encoding mCitricine alone; 4, NT2D1 hEC. (D) 1, mock transduced HeLa; 2, HeLa transduced with mCherry-Klf4-encoding vector at MOI 5; 3, HeLa transduced with all 4 reprogramming vectors at MOI 1; 4, NT2D1 hEC; 5, mock-transduced BJ fibroblasts; 6, BJ fibroblasts transduced with mCherry-Klf4-encoding vector; 7, BJ fibroblasts transduced with all 4 reprogramming vectors; 8, mock-transduced MRC-5 fibroblasts; 9, MRC-5 fibroblasts transduced with all 4 reprogramming vectors. (E) MRC-5 cells transduced with the vector encoding vexGFP-Oct4 at MOI 30, 10, 3, 1, as indicated. UT, untransduced.

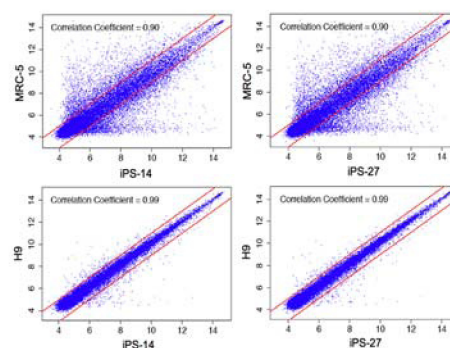
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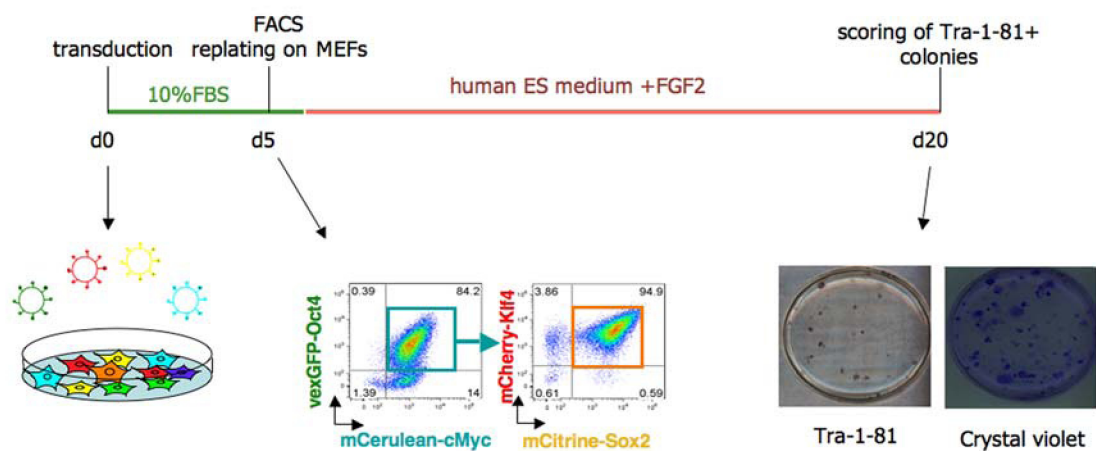
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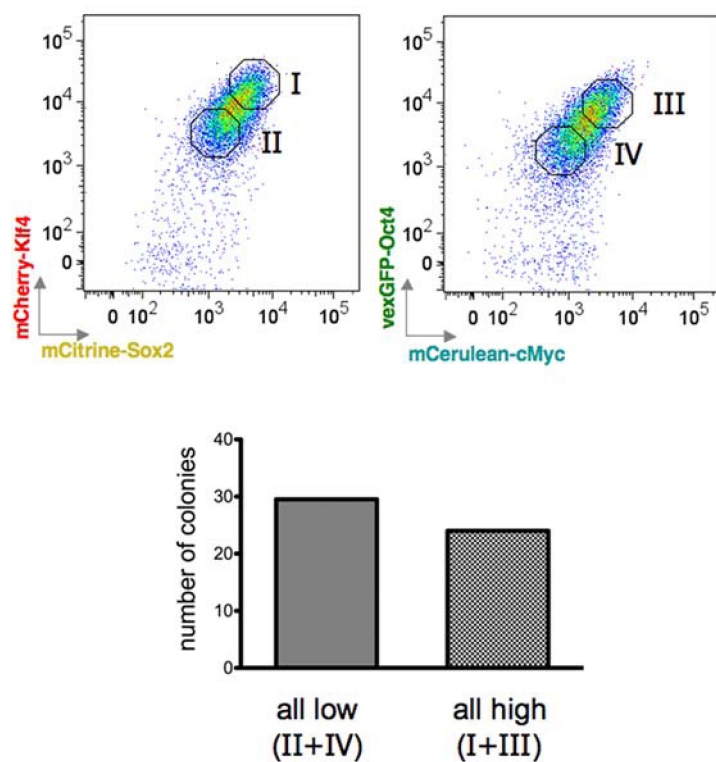
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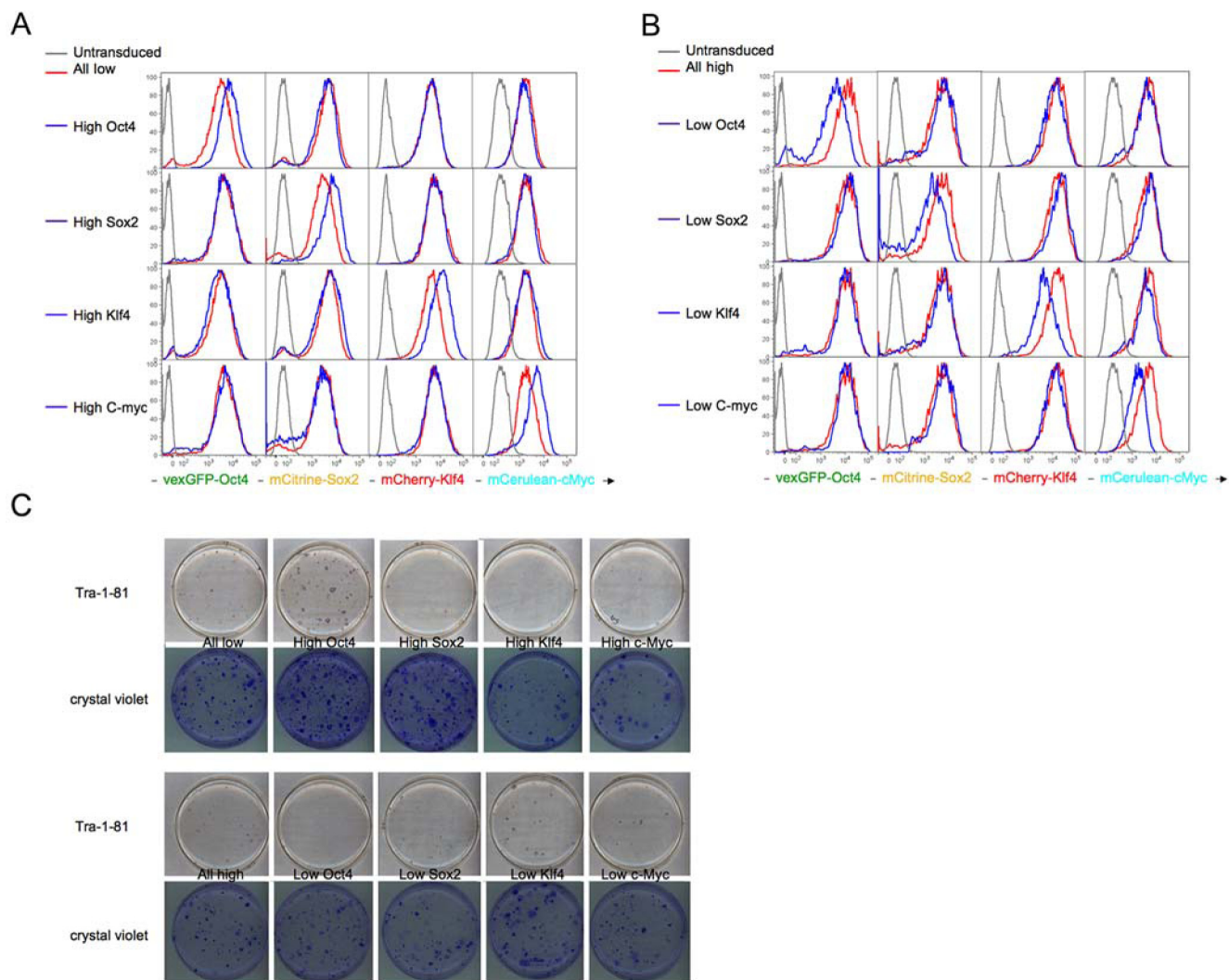
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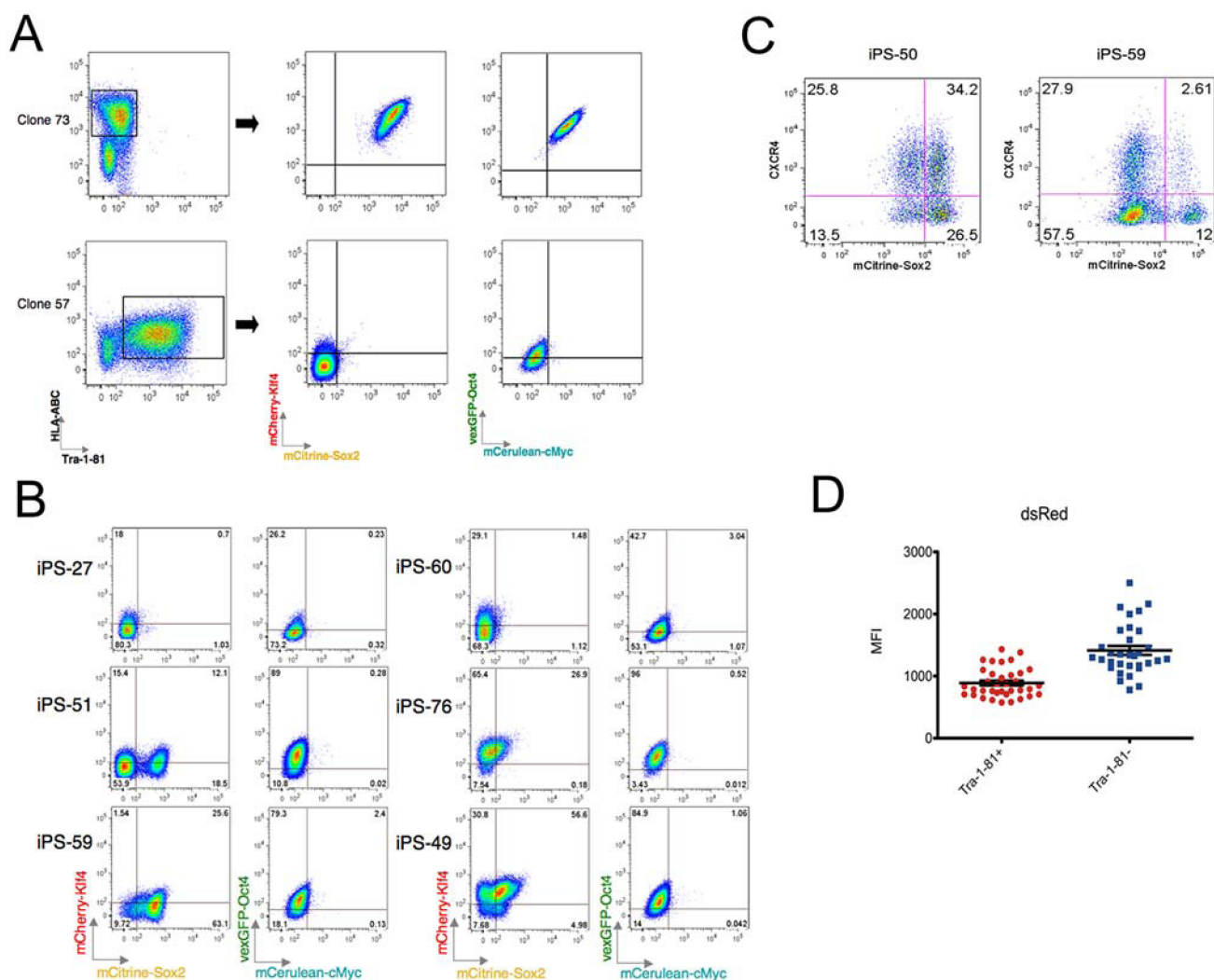


**Fig. S2.** (A) Immunofluorescence staining of hiPSC line 14 (iPS-14) for Tra-1-81, Tra-1-60, SSEA3, and Nanog. (B) In vitro differentiation of hiPSC lines 14 (iPS-14) and 27 (iPS-27) into endoderm, mesoderm, and neuroectoderm, followed by staining for Sox17, Brachyury, and Pax6, respectively. (C) Microarray analysis of whole genome gene expression of iPS-14, iPS-27, hESCs (line H9), and MRC-5 fibroblasts. (D) The experimental strategy used in this study for accurate calculation of reprogramming efficiency. Human fetal fibroblasts are transduced with the 4 reprogramming vectors shown in Fig. 1A on day 0. On day 5 the cells are harvested, counted and replated on MEFs, while a fraction is used for quantification of the fraction of quadruple positive cells by flow cytometry. Tra-1-81+ colonies are scored on day 20 as described in the *Methods*.

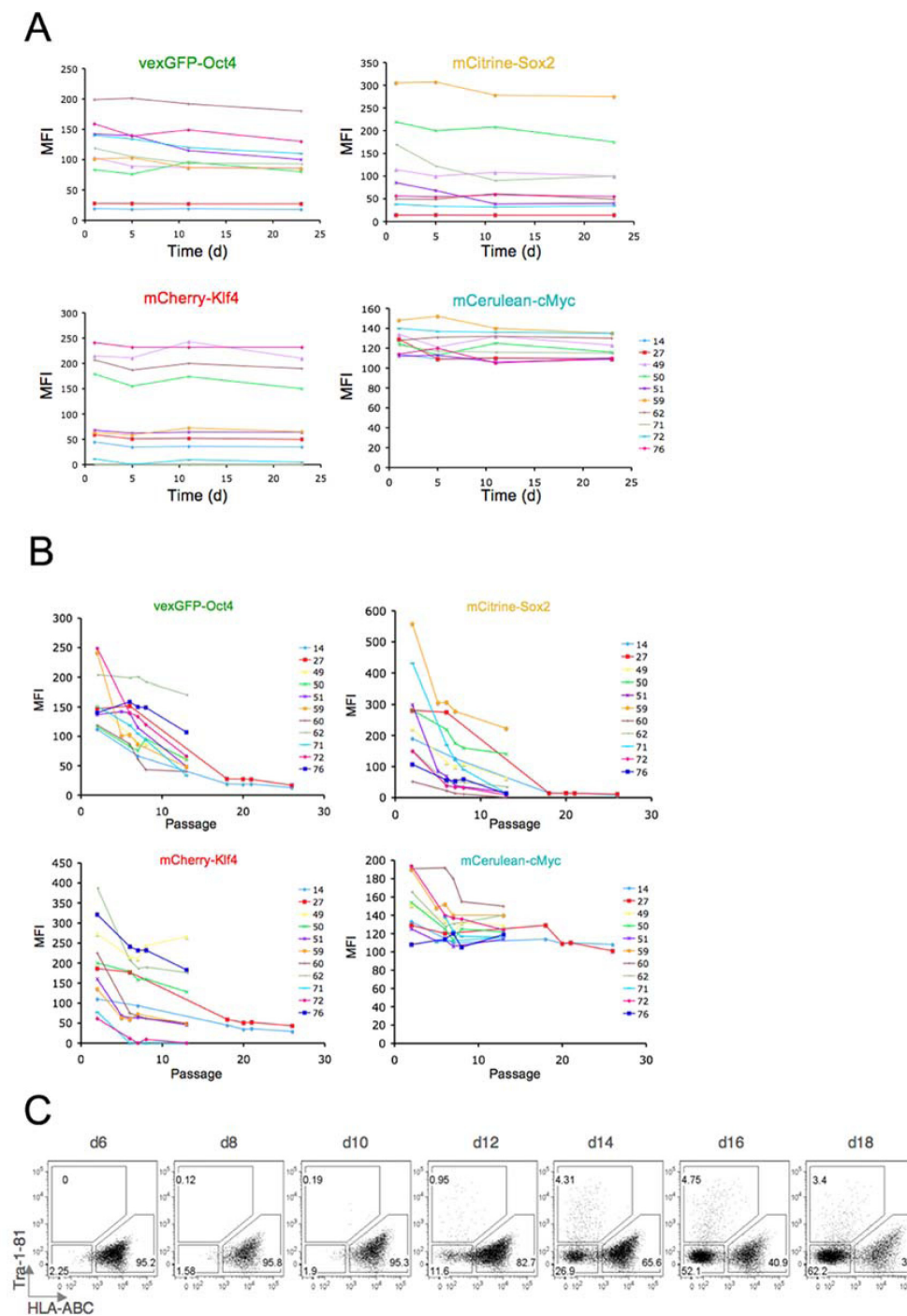


**Fig. S3.** Reprogramming efficiency of FACS-sorted MRC-5 fibroblasts expressing low or high levels of all 4 RFs. *Top*, sorting strategy. *Bottom*, number of Tra-1-81+ colonies per 10,000 cells (y axis) derived from cells expressing low (sorted as II+IV) or high (sorted as I+III) levels of all 4 RFs.





**Fig. S5.** (A) Flow cytometry analysis of vector expression in a representative fully reprogrammed (clone 57) and a non-hiPSC clone (clone 73). (B) Flow cytometry analysis of vector-encoded transgenes in undifferentiated hiPSC clones 27, 51, 59, 60, 76, and 49. Numbers within plots denote percentage of cells in the respective quadrants. (C) Directed differentiation of hiPSC clones 50 and 59 into endoderm, followed by flow cytometry analysis. Numbers within plots denote percentage of cells in the respective quadrants. (D) dsRed expression in 36 Tra-1-81+ (red circles) and 32 Tra-1-81- (blue squares) clones derived from MRC-5 fibroblasts co-transduced with the 4 reprogramming vectors and an additional dsRed-encoding vector.



**Fig. S6.** (A) Time course of vector-encoded factor expression (as assessed by MFI of the corresponding fluorescent protein, shown in y axis) in hiPSC lines 14, 27, 49, 50, 51, 59, 62, 71, 72, and 76 upon 21 days of induction of neuronal differentiation (Day 0 denotes the beginning of neural induction as described in the *Methods*). No upregulation or re-activation of the vector-encoded transgenes is observed. (B) Time course of vector-encoded factor expression (as assessed by MFI of the corresponding fluorescent protein, shown in y axis) in hiPSC lines 14, 27, 49, 50, 51, 59, 60, 62, 71, 72, and 76 maintained in undifferentiated conditions over several passages (x axis). Gradual decrease in vector-encoded factor expression is observed with increasing passage. (C) Flow cytometry time course of Tra-1-81 and HLA-ABC expression during reprogramming. Dot plots shown are pregated on quadruple transduced cells. Numbers within plots denote percentage of cells in the respective gates.

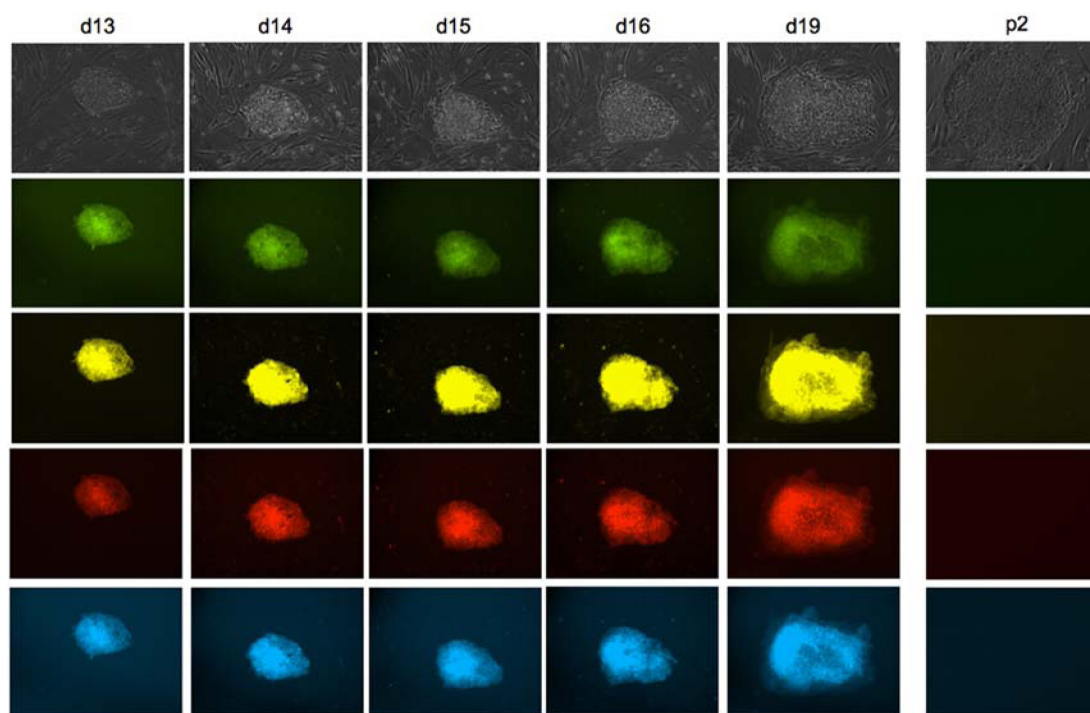


Fig. S7. Imaging time course of hiPSC clone iPS-14 at days 13–19 after transduction, as indicated, as well as in passage 2 (p2).

## Other Supporting Information Files

[Table S1](#)

[Table S2](#)